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THE SKIN MICROFLORA IN ACNE VULGARIS*

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ABSTRACT

Fifty-eight patients with acne were studied in regard to the microbial flora of the scalp, forehead, cheek, anterior nares, and open and closed comedones. Males carried higher numbers of aerobic bacteria and *Pityrosporum* at all sites. Patients with inflammatory acne also supported larger populations of these organisms in comparison to those with dominantly comedonal disease. *Corynebacterium acnes* density was not clearly affected by sex or type of disease. Group II *C. acnes* occurred most frequently in the nose and in comedones. Greater densities were found in patients with inflammatory disease. Bacteriophage against group I *C. acnes* was recovered from all but three patients.

Three groups of microorganisms are regularly recovered from the lesions of acne vulgaris: *Corynebacterium acnes*, *Staphylococcus epidermidis* and *Pityrosporum* [1-3]. The same organisms are found on the facial skin of persons without acne; thus their potential significance in pathogenesis is unclear. Current thinking implicates *C. acnes* etiologically while generally dismissing cocci and yeast-like fungi [4]. The proportion of free fatty acids in the surface lipids correlates well with the density of *C. acnes* [5, 6]. Free fatty acid levels invite attention because they are both comedogenic and irritating [7, 8]. Theoretically, patients with severe inflammatory acne ought then to have more *C. acnes* and more free fatty acids than patients with milder comedonal acne. Cotterill and colleagues, however, found that the proportion of free fatty acids was actually lower in males with highly inflammatory acne despite high sebum secretion rates [9].

Most previous work has focused on a single factor. We have undertaken in this study to examine the relationship between the quality of the disease and several variables, namely, sex, quantity of aerobes, *Pityrosporum*, *C. acnes*, and finally free fatty acids in the surface lipids.

MATERIALS AND METHODS

Patients

Fifty-eight patients—38 males and 20 females, ages 13 to 26 (median 17 years)—were selected at random from new patients attending an acne clinic at the Hospital of the University of Pennsylvania. Treatment was stopped at least one week before sampling. Some had received antibiotics previously. No patient had received any systemic or topical antibiotic therapy in the month prior to sampling.

Clinical Assessment

The patients were allocated to two groups on the basis of the predominant type of lesions, either inflammatory or comedonal acne. Patients with inflammatory acne had rather severe disease but none would be classified as acne conglobata.

Microbial Samples

Samples were taken from the scalp, forehead, and cheek by the detergent-scrub technique [10]. An effort was made to avoid regions with many lesions. Five open and 5 closed comedones were separately pooled and homogenized in 2.5 ml of Triton X-100 in phosphate buffer. A detergent-moistened swab was used to sample the anterior nares then returned to 2 ml of the detergent solution [1]. The mean densities were logarithmically transformed for statistical analysis.

Free Fatty Acids

Surface lipids were collected by 2 ether washes within a glass cup 3.8 sq cm in area. The solution was passed through a membrane, filter and the solvent removed under nitrogen. The proportion of free fatty acids was determined by thin-layer chromatography [12].

Bacteriologic Methods

Because quantitative cultures of *Pityrosporum* are not possible, the number of organisms was estimated directly on coverslips as previously described [13, 14].

*Single drops from serial tenfold dilutions in half-*strength wash fluid were inoculated on to trypticase soy agar, with and without lecithin and Tween 80 for quantitating aerobes. These were incubated for 2 days at 35° C. Modified Marshall and Kelsey agar [3] was similarly inoculated and incubated anaerobically for 7 days at 35° C to estimate *C. acnes*. The swab from the anterior nares was agitated mechanically in 2 ml of wash fluid and then serially diluted. Phenylethanol blood agar and MacConkey's agar were also inoculated and aerobically incubated to facilitate recovery of nonresident organisms. Comedo homogenates were serially diluted and plated as above.

Identification of colonies was made on streak plates of

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Marshall and Kelsey's medium incubated aerobically and anaerobically. To detect *C. acnes* bacteriophage, the fluid remaining from the first three dilutions and, for the nose only, the fourth, was passed through a 0.45-μ membrane filter. Single drops (1/32 ml) were placed on prepared lawn plates of group I *C. acnes*.

Organisms in the following groups were counted:

S. aureus

Coagulase negative cocci

Lipophilic diphtheroids

Miscellaneous diphtheroids

Gram-negative rods. These were further identified to the generic level [15].

C. acnes. These were separated in group I and group II on the basis of colony morphology and bacteriophage susceptibility. Identification of representative strains was confirmed by biochemical tests.

Pityrosporum was counted directly.

RESULTS

Flora of Different Sites

The geometric mean densities and 95 percent confidence limits of aerobes, *C. acnes*, and *Pityrosporum* for each of the sites are shown in Figure 1. Table I breaks these down further in relation to sex and type of disease. On the scalp more than a million organisms per sq cm of both *C. acnes* and *Pityrosporum* were found. The count of aerobic bacteria was somewhat lower, 300,000 organisms per sq cm, practically all of which were cocci.

The same organisms were found on the forehead and cheek but the densities were proportionately lower. More *C. acnes* were recovered from the cheek. The aerobic flora again was predominantly coccal.

The nose carried a distinctly different flora. There were fewer *Pityrosporum* and *C. acnes*. The aerobic bacteria dominated and the composition was complex. Forty percent were lipophilic diphtheroids. *Staphylococcus aureus* and gram-negative rods were also rather frequent but not in high numbers.

Comedones contained more *C. acnes* than aerobes or *Pityrosporum*, about 300,000 *C. acnes* for closed and open ones. The number of cocci was respectively 84,500 per open comedone and 79,500 per closed one—an insignificant difference. *Pityrosporum* numbered 136,000 per open comedone but only 55,200 in the closed type—a significant difference ($p < 0.01$). *S. aureus* was recovered in low numbers from the nose in 12 of 56 (21%) samples. One patient yielded a thousand *S. aureus* from the forehead only. One comedo harbored a million *S. aureus* and 2 other skin samples from nasal carriers showed low numbers. In only one subject was *S. aureus* found on the skin in the absence of nasal carriage.

Gram-Negative Rods

In the nose, 59 percent of the samples yielded one of more genera of gram-negative rods. The commonest were *Enterobacter* (12 samples), *Klebsiella* (10), *Escherichia* (6), and *Proteus* (3). Other gram-negatives were isolated 7 times. This 59 percent should be compared to 40 percent in an unselected group of normal subjects and 70 percent in acne subjects being treated with antibiotics [11]. As before, the numbers were small. In 170 samples, gram-negative rods were rare on the skin, being recovered in small numbers, 3 times on the scalp and 5 each on the forehead and cheek. *Enterobacter* was the usual genus. On the other hand, 15 of 58 pooled comedones yielded gram-negatives. *Enterobacter* was commonest (11 times), *Klebsiella* occurred twice and *Escherichia*, *Pseudomonas*, and *Mima* once each. The numbers were small, but in a few cases the density was more than 10^4 or about 5 percent of the flora. The highest level was 60,000 *Enterobacter* in a total population of 530,000.

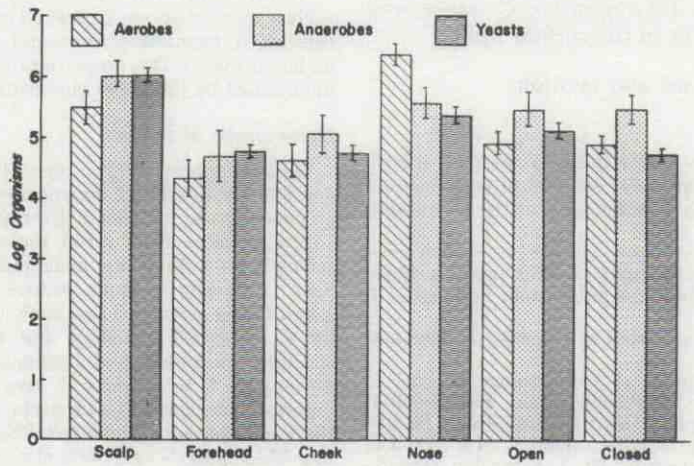

FIG. 1: Regional variation in the cutaneous fina of 58 acne patients. The bars represent the 95 percent confidence limits of the standard error of the mean.

TABLE I

Geometric mean microbial density in acne patients with inflammatory (I) and comedonal (C) disease

Site				Aerobes	*C. acnes*	I*	%	*C. acnes* II (in carriers)	Pityrosporum
Scalp (per sq cm)	Males	I	24	653,600	823,300	7	29	16,590	1,374,000
		C	12	158,100	1,710,000	7	58	6,445	708,600
	Females	I	6	374,500	1,295,000	3	50	6,542	1,407,000
		C	13	132,200	1,095,000	3	23	2,105	764,300
	Total		55	309,000	1,085,000	20	36	7,600	1,038,000
Forehead (per sq cm)	Males	I	25	37,630	13,060	9	36	7,536	70,110
		C	13	9,283	70,960	8	51.5	863	58,250
	Females	I	7	116,100	423,100	2	29	4,236	86,260
		C	13	7,251	159,900	4	38	4,469	41,560
	Total		58	21,800	49,900	23	40	3,080	61,000
Cheek (per sq cm)	Males	I	24	105,700	118,800	9	37.5	41,360	68,920
		C	13	29,630	183,700	6	46	19,150	73,400
	Females	I	7	128,200	206,100	1	14	2,105	68,940
		C	13	6,390	56,300	2	15	9,647	29,250
	Total		57	42,700	118,800	18	32	24,600	57,500
Open (per lesion)	Males	I	24	130,200	358,000	19	79	84,800	143,000
		C	12	90,760	682,000	9	75	29,690	140,800
	Females	I	7	47,200	204,800	4	57	5,939	173,800
		C	10	41,420	93,850	6	60	5,667	99,550
	Total		53	85,000	258,000	38	72	33,000	136,000
Closed (per lesion)	Males	I	24	80,000	375,500	19	79	68,250	61,370
		C	12	79,000	403,500	9	15	31,300	66,700
	Females	I	7	104,900	332,100	5	71	68,280	59,260
		C	10	65,720	121,000	7	70	20,450	34,000
	Total		53	79,800	300,000	40	75	46,000	55,000
Nose (per sample)	Males	I	25	3,633,000	339,800	22	88	108,600	262,600
		C	12	1,567,000	560,700	11	92	63,840	275,600
	Females	I	6	1,346,000	195,500	5	83	60,130	311,100
		C	13	1,683,000	397,900	9	69	17,730	160,100
	Total		56	2,272,000	371,000	47	84	82,200	237,000

* I = Incidence of group II *C. acnes*

Bacteriophage

C. acnes group I bacteriophage was detected in at least one site from all but three patients. Phage was most numerous in the anterior nares where up to 10^7 plaque-forming units occurred (median 10^5). Forty-two of 54 samples yielded phage, but usually the numbers were small, the median being only 10^2. Only 38 percent of comedones yielded phage, but the numbers were often moderately large, with a mean of 10^3. There was no difference in relationship to the type of disease, but females carried phage at a significantly lower frequency than males ($X^2 = 6.14$, $p < 0.05$).

Type of Disease and Sex

Table I shows the microflora in relation to disease type and sex. There were more males then females, (38 to 20). The incidence of inflammatory acne was greater in males, 25 of 38 males, 7 of 20 females ($X^2 = 3.86$, $p < 0.005$). Two trends could be noted. Firstly, patients with inflammatory acne carried more organisms of all types than patients with comedonal acne. Secondly, males appeared to carry more organisms than females. By summating all the results (all sites, all organisms), these trends were found to be statistically significant. The average density of organisms in inflammatory acne exceeded that of patients with comedonal acne in 36 of 45 ($X^2 = 5.38$, $p < 0.05$). Males exceeded females in 20 of 24 comparisons within comedonal acne, but in only 13 of 24 comparisons within patients with inflammatory acne, a significant deviation from the number expected ($X^2 = 10.8$, 3 df, $p < 0.05$). The density of each of the main groups of organisms was separately determined. In all of the analyses significant differences between the sites were detected.

Aerobic Bacteria

Figure 2 depicts graphically the data in the first column of Table I. Significant differences between patients with inflammatory and comedonal acne could be detected in all three skin sites in males, but only on the forehead and cheek in females. Males carried significantly more aerobes in open comedones irrespective of type of disease, but this was not statistically verifiable in closed comedones. In the nose, males with inflammatory acne carried the most bacteria, but the difference was just within the 5 percent level.

C. acnes

Males carried more *C. acnes* in lesions and in the nose, but there was no clear pattern on the skin. The forehead showed anomalously low *C. acnes* counts in males with inflammatory acne.

As regards the two types of *C. acnes*, group I was most prevalent and most numerous. In the nose, however, 19 of 56 (34%) samples yielded only group II *C. acnes*, perhaps reflecting the destruction of group I by bacteriophage. Of the remaining 266 samples, group I *C. acnes* was present in high densities in all but 10. Group II *C. acnes* could not be considered universal, being isolated from 64 percent of the samples from males, but only 46 percent of the samples from females ($n = 322$, $X^2 = 8.97$, $p < 0.001$). Carriage was most frequent in the nose and in comedones and least frequent on the skin. Densities were too low and carriage too infrequent on the skin areas to draw any firm conclusions, yet the pattern of higher densities in males and also in inflammatory acne could be discerned. In the comedones, only open comedones showed a sex difference while closed comedones showed a considerable difference between inflammatory and comedonal disease groups in both sexes. In the nose both effects could be seen. Patients with inflammatory acne yielded group II *C. acnes* at the same frequency as comedonal acne patients (58% vs 65%), yet greater densities were present in inflammatory acne over all sites.

Pityrosporum

Patients with inflammatory acne carried more *Pityrosporum* on the scalp ($p < 0.05$), on the forehead, in the nose, in comedones, but not on the cheek in males. The two sexes could not be distinguished with regard to the density of *Pityrosporum*.

Free Fatty Acids (FFA)

On the forehead the range of free fatty acids was from 5 to 38 percent. A significant correlation ($r = 0.57$, $p < 0.01$) was found between the density of *C. acnes* and the percentage of FFA in the surface lipids. Figure 3 shows the regression line of FFA on *C. acnes*. (A square root transformation was applied.) The correlation also held for the cheek ($r = 0.50$). Here there was also a correlation between *Pityrosporum* and FFA ($r = 0.47$, $p < 0.01$), but not on the forehead ($r = 0.24$). Table II shows the relation of FFA to sex and type of acne. Males and females did not differ significantly in respect to FFA. The trend was towards higher values in inflammatory acne in both sexes. By analysis of variance this was significant ($p < 0.05$).

DISCUSSION

Previous studies have indicated that aerobic gram-positive bacteria play little or no role in the pathogenesis of acne. Although the cocci may produce lipase in vitro, their virtual elimination by topical antibiotics has no effect on the FFA level. The lipid-dependent yeasts appear to be secondary colonists of comedones but probably have no etiologic role [3]. Most interest attaches to *C.*

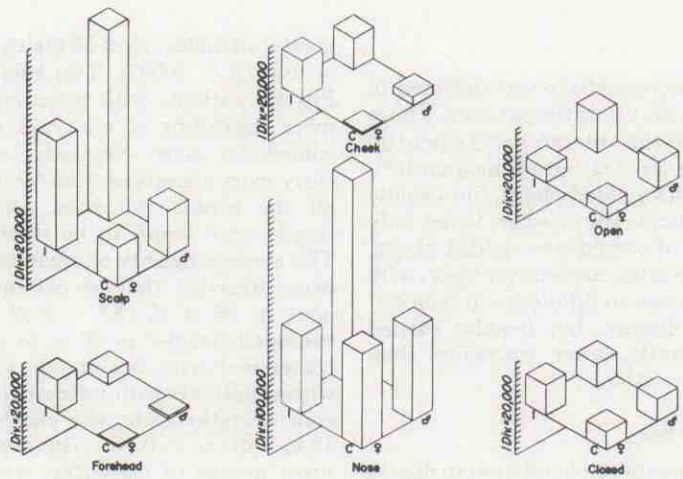

FIG. 2: Graphic representation of the first column in Table I. Patients with inflammatory acne carry much higher densities of aerobic organisms. Males in general also carry higher numbers of aerobes.

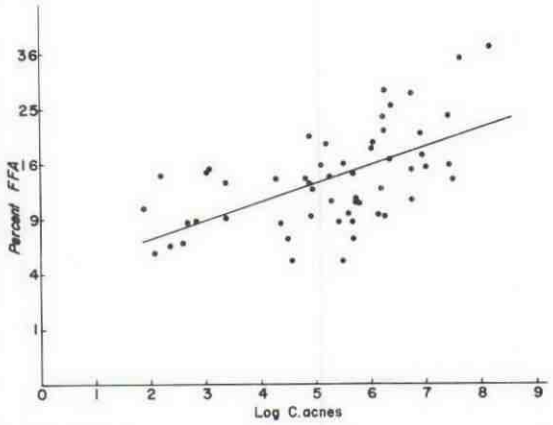

FIG. 3: Correlation of density of *C. acnes* in the forehead with surface free fatty acids ($r = 0.57$, $p < 0.01$).

TABLE II

Mean free fatty acid levels

		Sample	Forehead	Cheek
Males	I	25	14.77%	17.65%
	C	12	11.35%	16.27%
Females	I	7	15.68%	12.17%
	C	13	12.57%	11.65%
Total	I	32	14.97%	16.36%
Total	C	25	11.98%	13.77%

acnes, for various lines of investigation implicate this organism in pathogenesis. Its dominant role in liberating irritant and comedogenic FFA from triglycerides is now beyond dispute [4, 5, 7, 8, 16, 17].

In this study, quantitative microbiologic methods were employed to determine whether differences in the microbial flora existed between patients with inflammatory acne and comedonal acne and whether differences could be related to sex. The principal findings were:

1. Patients with inflammatory acne harbored greater quantities of resident organisms than those with the comedonal type. This was especially evident with aerobic cocci and *Pityrosporum*.

2. Males tended to carry more organisms than females in simple comedonal acne while this difference was blurred in inflammatory acne.

3. The nasal flora was distinctly different than that of the face and scalp. Gram-negatives were frequently isolated but in small numbers. *S. aureus* and enterobacteria, although frequently colonizing the nose in small numbers, did not form a part of the flora of the skin.

4. On the forehead and cheek, the proportion of free fatty acids in the surface lipids correlated with the density of *C. acnes*.

The finding of greater numbers of organisms on the face of subjects with inflammatory acne is not at all surprising. Exudation and increased moisture would promote bacterial growth, especially of surface residents rather than a follicular inhabitant like *C. acnes* as actually occurred. It is more difficult to explain why aerobes should be more numerous in the scalp and nose in inflammatory acne. Perhaps this indicates some basic anatomic characteristic which underlies the greater tendency towards more severe disease. Patients with inflammatory acne do have more irritable skins, in our experience, and their greater oiliness is an indication of larger follicles. Males can be expected to produce more sweat and sebum than females. This adequately accounts for higher densities of resident organisms in practically all sites.

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